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# The nervous system and metabolic dysregulation: emerging evidence converges on ketogenic diet therapy

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A link between metabolism and brain function is clear. Since ancient times, epileptic seizures were noted as treatable with fasting, and historical observations of the therapeutic benefits of fasting on epilepsy were confirmed nearly 100 years ago. Shortly thereafter a high fat, low-carbohydrate ketogenic diet (KD) debuted as a therapy to reduce seizures. This strict regimen could mimic the metabolic effects of fasting while allowing adequate caloric intake for ongoing energy demands. Today, KD therapy, which forces predominantly ketone-based rather than glucose-based metabolism, is now well-established as highly successful in reducing seizures. Cellular metabolic dysfunction in the nervous system has been recognized as existing side-by-side with nervous system disorders – although often with much less obvious cause-and-effect as the relationship between fasting and seizures. Rekindled interest in metabolic and dietary therapies for brain disorders complements new insight into their mechanisms and broader implications. Here we describe the emerging relationship between a KD and adenosine as a way to reset brain metabolism and neuronal activity and disrupt a cycle of dysfunction. We also provide an overview of the effects of a KD on cognition and recent data on the effects of a KD on pain, and explore the relative time course quantified among hallmark metabolic changes, altered neuron function and altered animal behavior assessed after diet administration. We predict continued applications of metabolic therapies in treating dysfunction including and beyond the nervous system.

**Keywords:** adenosine, epilepsy, glucose, inflammation, long-term potentiation, metabolism, pain, seizure

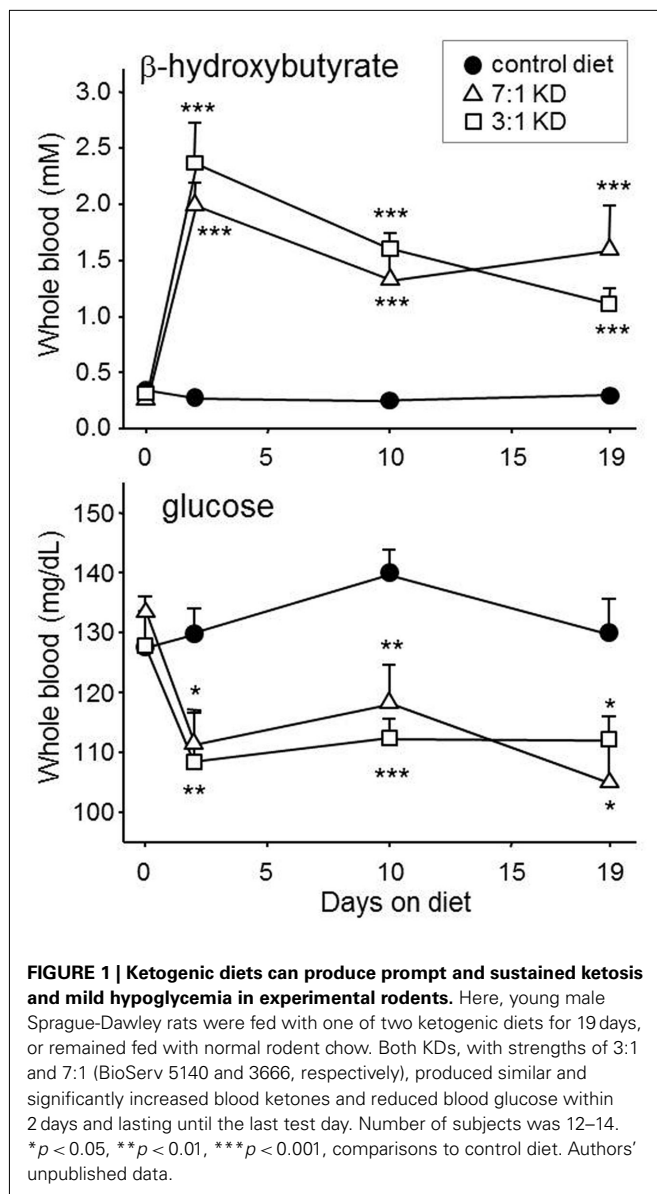
## THE KETOGENIC DIET AND KETONE-BASED METABOLISM

Metabolism influences brain activity, and metabolic dysfunction is associated with a wide variety of neurological disorders. The cause-and-effect relationship between metabolic and neuronal dysfunction is often unclear, though not in the case of epilepsy and diet. Historical observations noted the therapeutic benefits of fasting on epilepsy, but fasting is necessarily a time-limited practice. Therapeutic benefits of the metabolic condition of fasting were confirmed over 90 years ago when the high fat, low-carbohydrate ketogenic diet (KD) was described as alternative to fasting which still reduced epileptic seizures (Wilder, 1921). In turn, anticonvulsant drugs debuted over the next two decades, such that since then the KD has been used mostly for inoperable and medication-resistant epilepsy, which has been estimated to be 15% up to 45% of cases (Picot et al., 2008; Dong et al., 2011).

Although used clinically for many decades, prescribed most often to children, and increasing in popularity over the last two decades, the KD's mechanism of action remains controversial. The KD was designed to produce ketosis without fasting by strictly limiting carbohydrate intake (Wilder, 1921). To make up for lost calories and augment ketosis, fat content is increased dramatically. When carbohydrate intake is strongly limited (as during the KD or fasting), the liver increases production of the ketone bodies  $\beta$ -hydroxybutyrate, acetoacetate, and acetone from circulating fatty acids (Aoki, 1981). Because of

the  $\beta$ -hydroxyl substitution,  $\beta$ -hydroxybutyrate is not actually a ketone, although by convention it is grouped with the other two ketone bodies.

Ketone bodies are released into the circulation as an alternative energy source to generate ATP ("ketolytic" metabolism) within tissues, including the brain and spinal cord. Hallmark changes in blood chemistry are produced reliably in rodents (Figure 1). Formulation of the KD is calculated using a ratio of fat content to combined protein and carbohydrate content, varying in the clinic from 5:1 to 1:1 depending on a patient's individual needs (Swink et al., 1997; Vining, 1999). We found that a KD-fed *ad libitum* at ratio of 7:1 or 3:1 to rats produced similar changes in blood chemistry (Figure 1). Clinically, the trend has been to decrease the ratio where possible and thus make the diet more palatable (including the more liberal modified Atkins diet; Kang et al., 2007; Kossoff et al., 2008b) but more systematic research is needed. Regarding different food types, the KD has now been adapted for widely varying cultures and cuisines in different countries around the world (e.g., India, Korea, United Kingdom, Saudi Arabia, Republic of Georgia; Kang et al., 2007; Neal et al., 2008a; Sharma et al., 2009; B. Zupiec-Kania, personal communication). Understanding the mechanisms by which a diet controls seizures, along with broader opportunities for metabolic therapies, remains an active research topic because of accessibility, efficacy, and economics.



## METABOLISM, PLASTICITY, AND SYNAPTIC ACTIVITY

The KD might alleviate seizures and other pathological states partially by providing elevated levels of high-energy molecules (e.g., ATP, phosphocreatine) and increased capacity for energy generation (increased mitochondrial number; Seyfried and Mukherjee, 2005; Bough and Rho, 2007; Masino and Geiger, 2008). Yet, numerous other changes due to the KD have been hypothesized to underlie increased inhibition and/or decreased excitation in brain, and thus to an anticonvulsant/neuroprotective state. In normal humans fed a KD, electroencephalography and transcranial magnetic stimulation demonstrated increased inhibition in the cerebral cortex, with a magnitude similar to that seen after benzodiazepine administration (Cantello et al., 2007). With the more extensive investigation possible in experimental animals, a KD was shown to enhance paired-pulse depression, shift the input/output relationship rightward, elevate the threshold for maximal electrical

activation, and to block spreading depression-style events in the hippocampus *in vivo* (Bough et al., 2003). There have been surprisingly few detailed studies on detailed synaptic effects, likely because of the difficulty in performing such studies *in vivo*, coupled with the typical glucose-based incubation protocol for *in vitro* slices; to date, a “KD” incubation protocol has not been standardized, although recent work sampling cerebrospinal fluid in KD-fed animals might provide a starting point (Samala et al., 2011). Currently, the major proposed mechanisms for such increased inhibition and/or decreased excitation include increased levels of adenosine, a major inhibitory neuromodulator (Masino and Geiger, 2008); increased levels of  $\gamma$ -aminobutyric acid (GABA), a major inhibitory neurotransmitter (Yudkoff et al., 2007; Omote et al., 2011); decreased glutamate, a major excitatory neurotransmitter (Lund et al., 2009; Juge et al., 2010) and direct effects of elevated ketone bodies on ion channels (Ma et al., 2007).

Increased inhibition or decreased excitability, if sufficiently strong, might not only suppress seizures but also influence normal brain function. Many types of normal brain function, as well as recovery from injury, are thought to depend on synaptic plasticity, i.e., the malleability, either temporary or long-lasting, of the strength of neuronal communication (Davis et al., 1992; Goosens and Maren, 2002). Long-term potentiation (LTP) is a sustained increase in synaptic efficacy which can be observed in a number of brain regions including its original discovery site, the hippocampus (Bliss and Lømo, 1973; Bramham and Srebro, 1989; Clugnet and LeDoux, 1990; Bonci and Malenka, 1999; Mahon et al., 2004). Studies have linked metabolism and LTP (Potter et al., 2010); we and our collaborators characterized the effects of a KD on hippocampal LTP with the hypothesis that KD-related inhibition or reduced excitation might affect brain plasticity (Koranda et al., 2011). We recorded hippocampal signals through chronically implanted electrodes in freely moving rats. After 3 weeks on a 7:1 KD, baseline synaptic measurements were taken in the perforant path-dentate gyrus pathway and LTP was induced with tetanic stimulation and the response measured over the next 2 days. The KD had no significant effects on measures of short-term plasticity (paired-pulse depression, paired-pulse facilitation), and did not prevent LTP induction, whereas the magnitude of the potentiation was significantly smaller in KD-fed rats. The LTP magnitude remained lower in these rats out to the longest tested time point (48 h). As discussed below, cognitive effects of the diet are mixed in animals and overall positive in humans. In addition, it is important to note that 7:1 is a stronger diet ratio than that used clinically, animals used had never had seizures, and another paper looking at the KD on LTP *in vivo* in anesthetized animals did not find any differences (Thio et al., 2010).

To test the role of adenosine in the KD's ability to reduce seizures, we and our collaborators recently tested the effectiveness of a KD in a transgenic mouse with spontaneous hippocampal electrographic seizures due to adenosine deficiency. These mice overexpress the adenosine-metabolizing enzyme adenosine kinase (ADK) in brain (Fedele et al., 2005), and tonic levels of the endogenous inhibitor adenosine are therefore lower than normal. At baseline, seizures recorded with chronically implanted electrodes occur five times per hour, on average (Masino et al., 2011). After being fed on a 7:1 KD for 3 weeks, seizure frequency dropped almost

90%. This antiseizure effect depended on low glucose (seizures were restored by a peripheral injection of glucose), and activation of the adenosine  $A_1$  receptor subtype ( $A_1R$ ; seizure activity was restored by injection of a selective  $A_1R$  antagonist). Together, this evidence suggests that the KD exerts antiseizure effects by restoring adenosine levels and  $A_1R$  activation via a mechanism related to low glucose.

Further support for this idea is provided by transgenic mice lacking  $A_1R$ s. These mice also have spontaneous electrographic seizures in the hippocampus, but the KD has no effect on seizure frequency in  $A_1R$  knockout mice, and is partially effective in mice heterozygous for the  $A_1R$  (Masino et al., 2011). Although these models all involve seizures induced by a lack of adenosinergic modulation, the results are likely generalizable: adenosine has been found to be anticonvulsive/antiseizure in virtually every seizure model in which it has been tested (excepting  $A_1R$  knockout mice – providing further evidence for the primary anticonvulsant role of  $A_1R$ s). Adenosine in particular, and a KD in general, might offer more homeostatic “upstream” bioenergetic regulation of neuronal activity, and possibly long-term benefits on brain homeostasis, than highly specific drug therapies (Boison et al., 2011). Regarding LTP, previous results consistent with the involvement of adenosine in KD effects have shown that adenosine reduces LTP magnitude when present during induction (Mitchell et al., 1993; Costenla et al., 1999; de Mendonca and Ribeiro, 2000; Fujii et al., 2000a,b; Tabata et al., 2001; Zhang et al., 2004; Rex et al., 2005; but see Pascual et al., 2005) and, when applied after induction, promotes reversal of existing LTP (Huang et al., 1999; Fujii et al., 2000a). Yet, the lack of effects of the KD on input–output relationships and short-term plasticity seem to argue against the tonic involvement of adenosine (Koranda et al., 2011). Mechanism aside, the KD can limit excessive neuronal activity (a class into which the neuronal activity during an LTP induction burst certainly applies) and perhaps reset baseline activity.

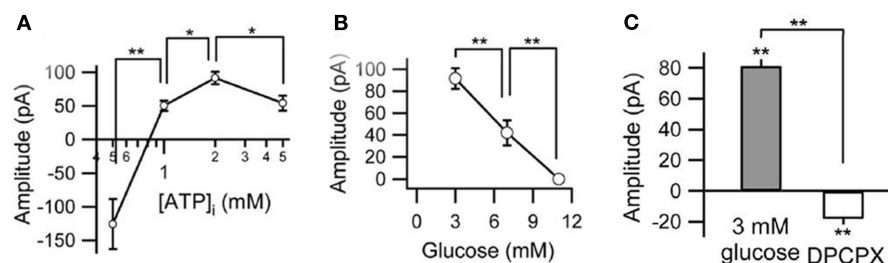
### KETOGENIC DIET FOR A BRAIN SLICE: RELAXING IN REDUCED GLUCOSE?

Compared to *in vivo*, *in vitro* paradigms can provide tighter control over experimental variables, allowing for a more thorough characterization of mechanisms. Effects of KD feeding on baseline excitability are inconsistent *in vitro*, however (Stafstrom et al.,

1999; Thio et al., 2000; Bough et al., 2006; Nylen et al., 2008). Certainly, the metabolic state established by a KD might be disrupted during tissue preparation for *in vitro* work. As introduced briefly above, one of the biochemical effects associated with a KD is an abundance of high-energy molecules (DeVivo et al., 1978; Nakazawa et al., 1983; Pan et al., 1999; Masino et al., 2007), as well as increased mitochondrial biogenesis, respiration, and expression of ATP synthesis-related proteins (Noh et al., 2004; Sullivan et al., 2004; Bough et al., 2006; Nylen et al., 2009; Ballester et al., 2010). Several lines of evidence suggest that reduced glucose is critical for antiseizure effects.

We modeled key aspects of the KD *in vitro* by maintaining or increasing intracellular ATP while decreasing extracellular glucose in individual CA3 pyramidal neurons in acute hippocampal slices. We varied ATP (0.5–5.0 mM; 2 mM is standard) in the patch pipet and changed glucose concentration of the bathing solution from 11 mM (standard) to either 7 or 3 mM (Kawamura et al., 2010). Note that 3 mM glucose is still a physiological level: *in vivo* brain concentrations are near 3 mM (Hu and Wilson, 1997; Shram et al., 1997). Moderately lowered extracellular glucose has been reported to attenuate epileptiform activity in brain slices (Kirchner et al., 2006), whereas experimental studies of pathological hypoglycemia often remove glucose completely from the bathing medium (aglycemia; Tromba et al., 1992; Zhu and Krnjevic, 1993).

We found that when intracellular ATP levels were adequate or high (1.0–5.0 mM), reducing extracellular glucose provoked an outward (inhibitory) current, with a larger current found with a reduction to 3 mM versus to 7 mM (Figure 2). This outward current was fully reversible on return to 11 mM glucose and had a reversal potential near the equilibrium potential for  $K^+$ , and was blocked by the non-selective  $K^+$  channel antagonist  $Ba^{2+}$  (Kawamura et al., 2010). If intracellular ATP levels were low (0.5 mM), reducing glucose produced a transient inward (excitatory) current instead (Figure 2). Therefore, moderately low extracellular glucose can inhibit hippocampal neurons that have sufficient or abundant energy stores. Furthermore, this inhibition was completely blocked by application of an  $A_1R$  antagonist and was not present in neurons from  $A_1R$  knockout mice (Figure 2; similar to observations *in vivo*: Masino et al., 2011) implying increased adenosine levels produced the inhibition (conversely, diabetic hyperglycemia seems to be related to reduced signaling through  $A_1R$ s (Duarte et al.,



**FIGURE 2 | Relationship among membrane current, intracellular ATP, and extracellular glucose. (A)** Peak currents produced by lowering extracellular glucose from 11 to 3 mM depend on intracellular ATP concentration (0.5–5 mM). Current is outward, except at 0.5 mM ATP. **(B)** Concentration-

dependence of glucose-related outward current (with 2 mM intracellular ATP). **(C)** Outward current produced in low-glucose buffer reversed completely (and became slightly inward) with the  $A_1R$  antagonist DPCPX. \* $p < 0.05$ , \*\* $p < 0.01$ . Adapted with permission from Kawamura et al. (2010).

2006). A similar consistent mechanism was reflected presynaptically (measured as decreased spontaneous postsynaptic current frequency); an  $A_1$ R-dependent presynaptic inhibition was produced by adequate/high postsynaptic intracellular ATP combined with low extracellular glucose (Kawamura et al., 2010). Together, this study and Masino et al. (2011) suggest that a KD can limit seizures (at least those involving the hippocampus) through a mechanism dependent on low glucose and abundant high-energy molecules and involving augmentation of adenosine levels.

In our *in vitro* study, we manipulated ATP only in the patched neuron, suggesting an autocrine mechanism to increase adenosine. How might this autoinhibition occur? ATP might be metabolized intracellularly to adenosine, which would then be released. Loading pyramidal neurons with adenosine + ATP versus ATP alone, however, suggested that the current was not mediated by direct adenosine release (Kawamura et al., 2010). Alternatively, ATP might be released and then metabolized to adenosine. Cells can release ATP by several mechanisms (Dubyak, 2009), and extracellular ATP is metabolized rapidly to adenosine (Dunwiddie et al., 1997). One prominent non-exocytotic ATP release mechanism in neurons and glia is ATP passage through channels composed of connexins or pannexins (Stout et al., 2002; Schock et al., 2008; Iwabuchi and Kawahara, 2011). Through a series of physiological and pharmacological experiments, we determined that pannexin channels were the source of extracellular ATP. Taken together, our data are consistent with a process by which lowered extracellular glucose promotes release of ATP via pannexins. ATP is then converted extracellularly to adenosine, which activates  $A_1$ Rs coupled, under these conditions, to  $K_{ATP}$  channels (Kawamura et al., 2010). This pathway is likely to underlie the  $A_1$ R-mediated anticonvulsant effect produced by the KD *in vivo*. Certainly, mild hypoglycemia and enhanced adenosine tone can underlie its anticonvulsant effect (Masino et al., 2011), whereas the *in vivo* involvement of pannexin channels and ATP release remains to be demonstrated directly.

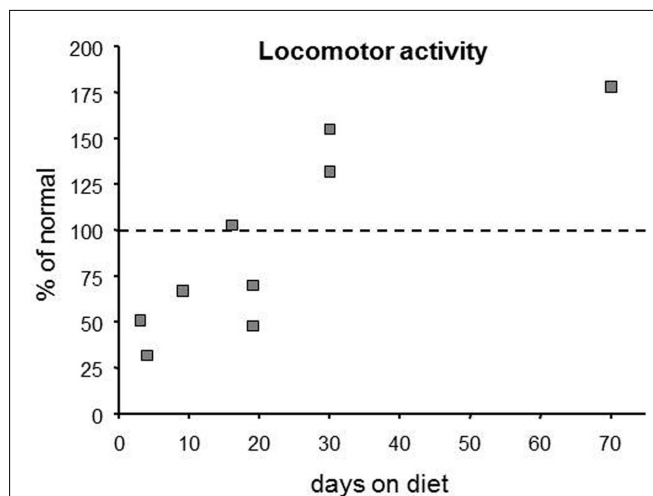
### KETOGENIC DIET'S EFFECT ON COGNITION AND MOOD: NEGATIVE, THEN POSITIVE?

Altered cognition and affect in children with seizure disorders has always been a concern. Regarding pharmacological therapies, several authors have shown that children with epilepsy – even those whose seizures were well-controlled with antiepileptic drugs – had decreased cognitive function compared to their peers (Devinsky, 1995; Thompson et al., 2000; Drane and Meador, 2002). The exact mechanism of cognitive decline is unknown: traditional antiepileptic drugs decrease membrane excitability, increase postsynaptic inhibition, or reduce network synchronization to decrease excessive excitability associated with seizure development (Loring, 2005). These neurophysiological mechanisms, if sufficiently strong, will not only suppress seizures but also impair normal brain function. The incidence of cognitive side effects is increased at higher dosing and with polypharmacy which might be necessary for significant seizure control (Loring and Kimford, 2001). Thus, the cognitive and affective state of a medicated epileptic patient results from a balance of forces including the negative effects of the disease state (seizures, abnormal interictal brain activity, abnormal sleep), the positive effects of the anticonvulsive medication

(seizure control), and the negative side effects of the anticonvulsive medication (which can include sedation and/or abnormal sleep).

The KD might offer fewer chronic negative side effects than medication, and given that it has been in use for over 90 years, serious or systematic negative consequences would likely have surfaced by now. In research studies, KDs (albeit at a much stronger ratio than used clinically) reduced brain mass in juvenile rodents (Cheng et al., 2004; Zhao et al., 2004) and KDs can affect body growth in children (who are typically on the diet temporarily; Liu et al., 2003; Peterson et al., 2005; Neal et al., 2008b) but to our knowledge negative KD effects on human brain development and growth have not been quantified. Notably, recurrent clinical hypoglycemia can lead to a cumulative cognitive impairment (Langan et al., 1991; Deary et al., 1993) – although this effect might not be directly applicable because the hypoglycemia in these studies was episodic and much more severe than the chronic reduced (but not abnormal) glucose levels associated with the KD. Overall, positive and negative short- and long-term effects of this strict diet on cognition and mood remain under-examined clinically, particularly in pediatric patients.

It is worthwhile to consider that any assessment of cognitive or affective state associated with a KD should occur at multiple time points, as effects of the KD (including anticonvulsive effects) clearly evolve. There are limitations to combining data from different laboratories due to differing methodologies, different KDs, etc. Yet in surveying the research literature, it seems fairly clear that there is a biphasic effect on locomotor behavior: reduced activity characterizes KD onset, whereas increased activity predominates after a few weeks. Effects of a KD on locomotion in rodents (compiled informally from the literature) are shown in Figure 3. Notably, a biphasic pattern over time after diet initiation



**FIGURE 3 | Over time, KDs produce a biphasic effect on locomotor activity based on this compilation of published rodent data.** Note that hypoactivity predominated in studies with short diet treatments ( $\leq 20$  day), whereas hyperactivity predominated with longer treatments ( $\geq 30$  day). Points were estimated from published graphs and tables in multiple references (Zhao et al., 2004; Murphy et al., 2005; Ziegler et al., 2005; Murphy and Burnham, 2006; Mantis et al., 2009; Oishi et al., 2010; Thio et al., 2010).



is found in clinical literature relating to cognition, mood, and vitality. Soon after beginning a KD, subjects often complain of lethargy (Vining et al., 1998; Lefevre and Aronson, 2000); in children, intolerable drowsiness is a reported side-effect that sometimes leads to cessation of KD treatment (Neal et al., 2008a). Yet, after weeks on the diet, subjects report heightened vitality, physical functioning, and alertness (Hallböök et al., 2007; Mosek et al., 2009; Yancy et al., 2009). In some cases these positive effects may be at least partially due to reduced seizure frequency, but similar positive effects are also described in non-epileptic subjects. This delay in beneficial effects is reminiscent of the delay often observed in anticonvulsant effects (Kossoff et al., 2008a).

Studies of the KD in epileptic patients rarely characterize mood, which might understandably be poor during the initial lethargic/drowsy stage. Several weight-loss studies, however, included affective measures and found positive effects of KD on mood in overweight subjects as early as 2 weeks into diet treatment, and lasting many weeks (Halyburton et al., 2007; McClernon et al., 2007; Brinkworth et al., 2009; Yancy et al., 2009). Two of these studies provide some evidence against this result simply being a psychological effect of weight loss (Brinkworth et al., 2009; Yancy et al., 2009). Thus, beneficial effects on mood (as well as weight loss) await those who conquer the early stage after KD initiation. Studies of patients with epilepsy on the KD, including children, have either reported improved cognition anecdotally (Sirven et al., 1999) or reported improvements in more general measures such as attention and social functioning (Kinsman et al., 1992; Pulsifer et al., 2001). It is difficult to determine if these effects are due to reduced seizures, to concomitantly reduced medications, or a direct action on cognition/attention. Investigations in non-epileptic adult subjects (thus without confounding antiepileptic medications) have more specifically addressed cognition and the KD. One study found a transient, moderate impairment in one cognitive task (but not two other tasks) at 1 week of diet treatment but found no impairments at later time points (Wing et al., 1995); two studies examining chronic KD treatments reported improved processing speed and working memory lasting up to 1 year (Halyburton et al., 2007; Brinkworth et al., 2009). This pattern seems to parallel the biphasic effect on activity and vitality noted above.

A minority of animal studies have reported impairments in learning and memory, specifically in a task of spatial reference memory (Su et al., 2000; Zhao et al., 2004). Other studies, however, have failed to find any detrimental effect of the KD on learning and memory in rodents in various mazes or in fear conditioning (Hori et al., 1997; Todorova et al., 2000; Silva et al., 2005; Appelberg et al., 2009; Thio et al., 2010). We tested normal mice of both sexes in a simple working memory task after feeding on a 7:1 KD at a number of time points, up to 10 weeks, and found no effect of the KD (though hyperactivity did appear beginning at 2 weeks (Ruskin et al., 2011a)). It is worth noting that a KD not only does not impair but in fact reverses age-related deficits in learning and other cognitive measures in aged, but otherwise healthy, dogs and rodents (Pan et al., 2010; Xu et al., 2010). Taken together, these results largely support the beneficial nature of KD feeding on mood and cognition in patients.

## NOCICEPTION AND INFLAMMATION: MULTIPLE MECHANISMS LIKELY

Converging lines of evidence suggest the utility of a KD for pain relief. First, it has long been known that reducing glucose metabolism influences pain. There is an overall increase in pain thresholds (and thus reduced pain) when glycolytic enzymes are inhibited by exogenous 2-deoxy-D-glucose (Bodnar et al., 1979). This effect is mediated centrally (Bodnar et al., 1981), and might involve increased brain/spinal cord inhibition by adenosine, the release of which is stimulated by 2-deoxy-D-glucose (Zhao et al., 1997; Minor et al., 2001). 2-Deoxy-D-glucose is also anticonvulsant (Garriga-Canut et al., 2006), and while the mechanisms might not overlap entirely with the KD (Stafstrom et al., 2009; Gasior et al., 2010) there might be some common pathways. Second, anticonvulsant drugs such as gabapentin, felbamate, and valproate are useful in treating pain, particularly neuropathic pain and migraine (Johannessen Landmark, 2008). These drugs typically act by decreasing neuronal activity or excitability, and it is clear that reducing central activity with adenosine or GABA agonists alleviates pain (Karlsten et al., 1992; Malmberg and Yaksh, 1993; Belfrage et al., 1995; Malan et al., 2002; Gwak et al., 2006). Thus, we predicted that the KD, which reduces glucose metabolism and is anticonvulsant, would reduce pain.

We fed rats a 7:1 KD in order to test the effects in the hot-plate test. In this test, the latency to withdraw a hindpaw from the warm surface indicates the animal's sensitivity to painful heat. In young rats, we found that KD feeding for 3–4 weeks increased paw withdrawal latency (i.e., decreased the sensitivity) to plate temperatures from 48 to 51°C (Ruskin et al., 2009). In adult rats, the effect seemed to be smaller in magnitude, and was significant only at 49 and 50°C. We recently found similar results with a less stringent 3:1 KD (Ruskin et al., 2011b). Curiously, another study reported increased thermal pain sensitivity (tail flick) after 12 weeks of KD feeding in young rats (Ziegler et al., 2005); methodological differences such as rat strain, body part (paw vs. tail), diet composition, and stimulus strength might be factors. The difference in diet treatment length (3 vs. 12 weeks) does not seem to explain the disparity, as subsequently we have found decreased thermal pain sensitivity present after 10–11 weeks of feeding with a 3:1 KD (Ruskin et al., 2011b). Thus far the specific mechanism of altered thermal nociception in KD-fed rats is unknown, and could involve hypoglycemia, ketosis, fatty acids, and/or adenosine.

One recently published clinical report on KD effects on “quality of life” reported that beneficial effects on self-reported general bodily pain were at the threshold of statistical significance (Yancy et al., 2009), suggesting that KD effects on overall pain might be positive. This report, however, was not a dedicated study of pain, but rather a study of overall quality of life; as such, there was no underlying painful condition to treat. In the same study, a low-fat diet also alleviated bodily pain. Overall, an assessment of pain in KD-treated patients is warranted.

A better understanding of the relationship between metabolism and pain could help multiple and comorbid conditions, and the KD might prove uniquely useful against diabetes and diabetes-related neuropathy. Although work with rodents has produced mixed results (Al-Khalifa et al., 2009, 2011; Garbow et al., 2011; Park et al., 2011; Poplawski et al., 2011), clinical

studies have found exclusively positive outcomes: after KD treatment, patients with type I or II diabetes had improved control of blood glucose, and many could have their medications reduced or eliminated (Gumbiner et al., 1996; Yancy et al., 2005; Westman et al., 2008; Dressler et al., 2010). In addition, type I diabetic patients (and, based on one report, children with epilepsy) prefer foods that are high in fat and low in carbohydrates (Amari et al., 2007; Snell-Bergeon et al., 2009), which might be attempted self-medication. The mixed animal results might result from the use of very strict KDs (Garbow et al., 2011; Park et al., 2011), or from the diabetic propensity of many laboratory rodent strains. Thus, the KD might benefit diabetic patients both by alleviating neuropathic pain and treating the underlying glycemic control dysfunction.

Finally, the KD would be predicted to be effective against inflammatory pain. Chronic inflammation is typically accompanied by pain due to the release of prostaglandins and the consequent sensitization of sensory neurons (Mense, 1983). Some of the most common sources of inflammatory pain are rheumatoid arthritis, chronic inflammatory bowel disease, pancreatitis, back pain, and some cancers. We found that a KD reduced experimental inflammation-induced swelling and plasma extravasation (Ruskin et al., 2009), and clinical studies describe positive effects of a KD on liver inflammation in non-alcoholic fatty liver disease (Tendler et al., 2007; Pérez-Guisado and Muñoz-Serrano, 2011). Regarding mechanisms linking metabolism to inflammatory pain, reactive oxygen species are a major component of inflammation, and limiting reactive oxygen species should contribute to limiting inflammation. Accordingly, ketone-based metabolism should produce fewer free radicals and reactive oxygen species through affecting the mitochondrial co-enzyme Q couple and the cytoplasmic glutathione couple (Veech, 2004). Indeed, as expected, treatment with ketones reduces the level of reactive oxygen species (Noh et al., 2006a; Kim et al., 2007, 2010; Maalouf et al., 2007; Haces et al., 2008; Maalouf and Rho, 2008), as does KD feeding (Sullivan et al., 2004).

Regarding inflammatory pain, by virtue of their high-fat content KDs should also activate peroxisome proliferator-activated receptors (PPARs). These nuclear receptors bind long-chain polyunsaturated fatty acids, and consequently induce transcriptional changes that culminate in enhanced lipid metabolism (Moya-Camarena et al., 1999; Diradourian et al., 2005; Michalik et al., 2006). Genetic knockout of a major PPAR (the  $\alpha$  subtype) augments inflammatory reactions (Cuzzocrea et al., 2006), whereas synthetic PPAR agonists reduce experimentally induced inflammation (Cuzzocrea et al., 2003; LoVerme et al., 2005). This latter effect appears to involve reduced transcription of pro-inflammatory genes (Blanquart et al., 2003) and seems to be invoked by the KD (Jeong et al., 2011). Synthetic PPAR agonists are analgesic against inflammatory pain (LoVerme et al., 2006). In addition to these effects, PPAR activation augments expression of the enzymes involved in ketogenesis (Cullingford et al., 2002), promoting the shift to a ketone-based metabolism, in agreement with findings of stronger ketosis with a high-polyunsaturated fat KD (Fuehrlein et al., 2004). Although polyunsaturated fatty acid content of the KD seems not to be important in the diet's anticonvulsant effect (Dell et al., 2001; Dahlin et al.,

2007), it might be a crucial characteristic for KD influence on inflammation.

It might seem ironic that the KD is discussed here as reducing inflammation, given that other high-fat diets and obesity are definitely linked to chronic inflammation (Thaler and Schwartz, 2010; Ding and Lund, 2011; Laugerette et al., 2011). Those high-fat diets that lead to obesity, including the so-called Western diet, include a high amount of fat along with normal amounts of carbohydrate, a crucial difference from the very low-carbohydrate KD which typically leads to weight loss (Gumbiner et al., 1996; Halyburton et al., 2007; Tendler et al., 2007; Westman et al., 2008). Thus, the high-fat-plus-carbohydrate diet promotes fat storage whereas the high fat, low-carbohydrate diet promotes fat metabolism. Nevertheless, more clinical work with the KD and inflammation is warranted, particularly regarding long-term effects. It will be crucial to determine which of the mechanisms described above is most important for the KD's alleviation of inflammation. Future work on the relationship between the KD's hallmark changes in blood chemistry, ketosis and mild hypoglycemia, and its anti-inflammatory and anti-nociceptive effects should help characterize the pertinent mechanisms.

## ATTENUATING BRAIN INJURY AND NEURODEGENERATION

Animal studies have found that the KD protects against seizure-induced neurodegeneration and related sequelae (such as aberrant neurite sprouting; Muller-Schwarze et al., 1999; Noh et al., 2003, 2005, 2006b; Linard et al., 2010). The KD is also neuroprotective against ischemic damage (Tai et al., 2008, 2009), hypoglycemic damage (Yamada et al., 2005), and traumatic brain and spinal injury (Prins et al., 2005; Appelberg et al., 2009; Hu et al., 2009a,b; Prins and Hovda, 2009; Schwartzkroin et al., 2010; Streijger et al., 2011), and improves injury-related deficits in cognition and movement after traumatic brain and spinal injury, respectively (Appelberg et al., 2009; Streijger et al., 2011). Ketosis is apparently crucial to these effects as direct application of ketones to *in vitro* tissue is also protective against hypoglycemia and ischemia (Samoilova et al., 2010), oxidative stress (Kim et al., 2007), and excitotoxicity (Massieu et al., 2003; Noh et al., 2006b; Maalouf et al., 2007; Samoilova et al., 2010). The mechanisms are likely to involve reduced reactive oxygen species, reduced tissue excitability, and enhanced production of high-energy molecules.

Based on evidence for neuroprotection against acute insults, and recognition that metabolic dysfunction accompanies chronic neurological disease, researchers are expanding into animal models of more slowly-acting neurodegenerative diseases. Positive effects of KD feeding have been found in models of amyotrophic lateral sclerosis (Zhao et al., 2006), Parkinson's disease (Cheng et al., 2009; Yang and Cheng, 2010), and Alzheimer's disease (Van der Auwera et al., 2005; Mohamed et al., 2010). In addition, KD feeding reverses aging-related impairments in brain biochemistry in animals (Studzinski et al., 2008; Balialetti et al., 2010). Direct application of ketones is also beneficial in models of Parkinson's disease (Kashiwaya et al., 2000; Tieu et al., 2003) and Alzheimer's disease (Kashiwaya et al., 2000).

Huntington's disease, which involves the death of neurons in the caudate and putamen, is thought to involve excitotoxicity and mitochondrial dysfunction (Estrada-Sanchez et al., 2008;

Damiano et al., 2010). Based on findings reviewed above, we characterized the effects of a strict (7:1) KD in a rapidly progressing Huntington's disease model, the R6/2 mouse (lifespan less than 16 weeks). KD feeding began at 6 weeks of age, when motor impairments are still minor (Ruskin et al., 2011a). The KD did not increase lifespan or alleviate motor impairments, but, importantly, it did not negatively affect either. However, the KD did delay significantly the onset of progressive weight loss, which is a major problem in patients (Sanberg et al., 1981; Lanska et al., 1988). In addition, the KD reversed a modest working memory impairment in female mice, and working memory is known to be affected in patients with Huntington's disease (Lange et al., 1995; Lawrence et al., 1996) as well as other neurological disorders and aging.

The lack of effect on lifespan or locomotor activity may signal that beneficial effects of a KD might not be similar across neurodegenerative disorders, might depend on the severity or rate of progression, or might differ in different animal models of a disorder; alternatively, the KD might need to be optimized for strength and composition for different conditions. Although it seems paradoxical that the KD, normally associated with weight loss, might maintain, or increase body weight under particular conditions, our data suggest that KD feeding could alleviate Huntington's disease-associated cachexia, and, as noted above, in patients a higher body mass is associated with slower disease progression (Myers et al., 1991). Based on this finding, the KD might also deserve consideration for treatment of other cachexias; for instance, that associated with cancer (Colomer et al., 2007). Indeed, the KD is

beginning to be used as an anti-tumorigenic treatment (Klement and Kammerer, 2011; Seyfried et al., 2012) and so could provide dual benefits. If the anti-neurodegenerative effects found in animal models of Parkinson's disease, Alzheimer's disease, and aging are successfully extended to humans, the KD could also have dual benefits, delaying the primary degenerative condition and alleviating the working memory problems common to these conditions (Halyburton et al., 2007; Brinkworth et al., 2009; Ruskin et al., 2011a).

## LOOKING AHEAD

A KD offers known benefits for epilepsy, and it is apparent that the relationship between metabolism and brain function offers primary therapeutic opportunities. Basic and clinical research is acutely aware that metabolic dysfunction and comorbidities promulgate lifelong impacts on nervous system function. Particularly promising unrealized opportunities for intervention and restoration of metabolic homeostasis occur during development, after injury, and during disease progression – all windows with high levels of plasticity and remodeling. New insight into mechanisms could accelerate development of treatments.

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